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PHYTOCHEMICAL STUDIES AND GC-MS ANALYSIS OF THE PLANT *ELEPHANTOPUS SCABER*

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ABSTRACT

This paper highlights the qualitative estimation of phytochemical compounds prese the methanol and aqueous extract of the plant *Elephantopus scaber* and also the GC analysis of the methanol extract of this plant. The qualitative analysis shows presence of proteins, carbohydrates, tannins, alkaloids, flavonoids, glycosides, ster saponnins and cholesterol in both the extract of the plant. The GC-MS analysis sl the presence of 9 bioactive compounds like 2-Amino-4-hydroxypteridine-6-Carbo acid with molecular weight 207, 25-Hydroxy-24-methylcholesterol with mole weight 560, Imidazole-2-aminovinyl-5-carboxylic acid with molecular weight 15 Methyl-2-Methoxy pyrazine with molecular weight 124, Arginine with mole weight 174, Methyl Jasmonate with molecular weight 224, Haloxazolam with mole weight 379, Asparagin with molecular weight 132 and Carbamic acid methyl present in the methanol extract of the plant *E. scaber*.

INTRODUCTION

The medicinal values of plants lie in their phytochemicals, which makes specific physiological actions on the human body. Phytochemicals are compounds found in plants that are utilized as food and medicine top reserve against illness and to ensure human health. Phytochemicals have antioxidant which helps in fighting against many diseases including cancer, heart disease, diabetes and high blood pressure (Prasad *et al.*, 2012). In developing countries, it is estimated that about 80% of the world population currently uses herbal medicine for some aspects of primary health care (Fransworth, 1993; Houghton, 1995). The importance of medicinal and aromatic plants has been emphasized from time to time due to their more safety and less side effects (Manish Devgun *et al.*, 2009; J.Srivastava *et al.*, 1996). Many conventional drugs or their precursors are derived from

plants. However, there is a difference between administering a pure isolated chemical and the same chemical in a plant matrix. Many higher plants accumulate extractable organic substances in quantities sufficient to be economically management of disease. Plants have been a rich source of medicines because they produce wide array of bioactive molecules, most of which probably evolved as chemical defense against predation or infection (Cox, P.A. and M.J. Balick, 1994).

*Elephantopus scaber L*inn is a small herb from the family Asteraceae, order used to treat diarrhoea, dysentery, stomach troubles and blood vomiting in tuberculosis in Nepal (Ahamed *et al*., 2009 and Ho *et al*., 2009)**.**

MATERIALS AND METHOD

PLANT COLLECTION

The plant materials were collected in the month of December, 2012 from the local areas. It was authenticated by Prof. Ramakanth Raju, Assistant Professor from Sri Vasavi institute of Pharmaceutical Sciences, Petadepalli, Tadepalligudem-534101, W. G. District from Andra Pradesh.

Extraction

The leaves of *Elephantopus scaber* Linn were dried under shade and then coarsely powdered. The powder was passed through sieve no.40 and stored in an air tight container for further use. The powder was then extracted with methanol and distilled water using Soxhlet apparatus for 72 hrs. The extract was dried and stored in dessicator. The extracts were subjected for chemical analysis by the standard procedures for identification of various phytoconstituents.

Qualitative phytochemical analysis

The methanol and aqueous extracts of the plant *Elephantopus scaber* were used to detect the presence of phytoconstituents such as carbohydrates, glycosides, alkaloids, proteins

Asterales and the subclass Asteridae. The whole plant of *Elephantopus scaber L*inn is well known as a herb of Chinese folk medicine which is widely used in the treatment of nephritis, edema, dampness, pain in the chest, fever and cough of pneumonia, scabies, and arthralgia due to wounding (Peer, 1980 and Tsai, 1999)**.** It is also commonly used in China as a remedy for the treatment of gastropathy, hepatitis, nephritis, edema, chest pain, fever and cough of pneumonia, bronchitis, arthritis, and carbuncle.

The root decoction of is widely tannins, saponins, flavanoids, triterpenoids and steroids.

Test for carbohydrates

Fehling's Test: To 1 ml of Fehlings A and Fehlings B solution were mixed and boiled for one minute. Then the equal volume of test solution (extract) was added to the above mixture. The solution was heated in boiling water bath for 5-10 minutes. Colour changed from yellow to brick red (Kokate, 1994).

Test for proteins

Xanthoproteic Test: To the small quantity of extract, 1ml of conc. H_2SO_4 was added, resulted in the formation of white precipitate which on boiling turned yellow. On addition of NH_4OH, yellow precipitate turned orange (Ansari, 2006).

Test for Glycosides

Keller-Killiani Test: To 2 ml of the extract, glacial acetic acid, one drop 5% $FeCl_3$ and conc. H_2SO_4 was added. Reddish brown colour appeared at junction of two liquid layers and upper layer turned bluish green indicating the presence of glycosides (Ansari, 2006).

Test for Steroids

Salkowski Test: To 2 ml of extract, 2 ml of chloroform and 2 ml of conc. H_2SO_4 was added. The solution was shaken well. As a result chloroform layer turned red and acid

layer showed greenish yellow fluorescence (IP, 1996).

Test for Alkaloids

The extract was evaporated in a test tube. To the residue dilute HCL was added, shaken well and filtered.

Mayer's Test: To the 2-3 ml of filtrate Mayer's reagent was added. Formation of yellow precipitate showed the presence of alkaloids (Ansari, 2006).

Test for Flavanoids

S. No	Tests	Methanol extract	Aqueous extract
1	Proteins	+	+
2	Carbohydrates	+	+
3	Alkaloids	+	+
4	Flavanoids	+	+
5	Glycosides	+	+
6	Tannins	+	+
7	Saponins	+	+
8	Steroids	+	+
9	Cholesterol	+	+

Shinoda Test: To the extract, 5 ml of 95 % ethanol and few drops of concentrated hydrochloric acid was added. To this solution 0.5 gm of magnesium turnings were added. Pink colouration indicated the presence of flavanoids (Kokate, 1994).

Test for Tannins

Lead Acetate Test: On addition of lead acetate solution to the extract white precipitate appeared (Mukherjee, 2002).

Test for Saponin

Foam Test: Drug extract was shaken vigorously with water. No persistent foam was formed (Ansari, 2006).

Test for Cholesterol

2ml chloroform was mixed with 10ml of plant extract to which 10-12 drops of acetic acid were added and shaken vigorously. There after 2 drops of conc. H_2SO_4 was added to change the colour from reddish brown to blue green (Harbone, 1998).

GAS CHROMATOGRAPHY-MASS SPECTROMETRY

About 5 gm of powdered material of plant was taken in a clean, flat-bottomed glass container and soaked in 25 ml of 80% methanol. The container with its content was sealed and kept for a period of seven days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through whatman filter paper. The filtrate (methanol and aqueous extract) obtained for the plant was evaporated under ceiling fan and in a water bath until dried.

GC-MS analysis was performed using The JEOL GCMATE II GC-MS with Data system is a high resolution, double focusing instrument. Maximum resolution: 6000 Maximum calibrated mass: 1500 Daltons equipped with a Elite-5MS (5% diphenyl/95% dimethyl poly siloxane) fused a capillary column (30 × 0.25 μm ID × 0.25 μm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/minute, and an injection volume of 2 μl was employed (a split ratio of 10:1). The injector temperature was maintained at 250° C, the ion-source temperature was 200° C, the oven temperature was programmed from 110° C (isothermal for

2 minutes), with an increase of 10° C/minute to 200° C, then 5° C / minute to 280° C, ending with a 9 minutes isothermal at 280° C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 2 minutes, and the total GC/MS running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas.

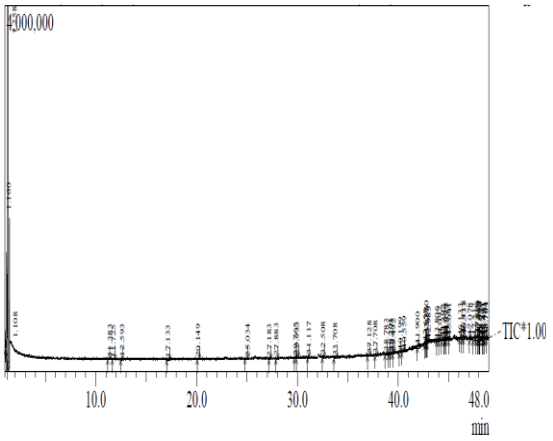
RESULT AND DISCUSSION

The qualitative phytochemical screening for the methanolic and aqueous extracts of the plant *Elephantopus scaber* were studied for proteins, carbohydrates, glycosides, alkaloids, tannins, saponins, flavonoids, steroids and cholesterol. The methanol and aqueous extracts shows the presence of all these compounds in the plant *E. scaber*.

Phytochemical studies of the methanol and aqueous extracts of *E. scaber*

The phytochemical qualitative analysis shows the presence of carbohydrates, proteins, alkaloids, flavonoids, glycosides, tannins, saponins, steroids and cholesterol in methanol and aqueous extract of the plant *E. scaber*, the presence of alkaloids, glycosides, cholesterol, flavonoids, steroids, and tannins in methanol and aqueous extract and the presence of saponins in the aqueous extract and the absence of saponins in the methanol extract of *E. scaber* (Kamalakannan *et al.,* 2012) also shows the presence of carbohydrates, proteins, tannins and saponins in the aqueous and methanol extracts, the presence of flavonoids in the methanol extract and the absence of flavonoids in the aqueous extract, but it shows the absence of alkaloids and steroids in both the extracts of the plant *E. scaber*. The variation in type of phytochemicals present in different solvents as shown in the result of phytochemical screening might be attributed to the ability of the solvents to dissolve into solution specific type of phytochemicals (Yusha *et al.*, 2008).

Moreover, alkaloids represent a class which affects the central nervous system, reduces appetite and behaves as diuretic (Dietary Guidelines for Americans, 2010). Numerous studies have confirmed that saponins possess the unique property of precipitating and coagulating red blood cells (Okwu. 2004, Sodipo *et al.*, 2000) and steroids are responsible for cholesterol-reducing properties. Steroids also help in regulating the immune response (Shah *et al.*, 2009). Tannins bind to proline rich proteins and interfere with the protein synthesis (Shimada, 2006).



Chromatogram of GC-MS analysis with the methanolic extract of the plant *Elephantopus scaber*

The compounds with their molecular formula and its percentage calculated from the chromatogram of GC-MS (methanolic extract of *Elephantopus scaber*) using NIST libraries

Sl. No	R.T	Name of Compound	Molecular Formula	Molecular Weight	Peak Area,%	Peak Height %
1	41.900	2-Amino-4-hydroxypteri dine-6-Carboxylic acid	$C_7H_5N_5O_3$	207	1.11	0.15
2	42.850	25-Hydroxy-24-methylcholesterol	$C_{34}H_{64}O_2Si_2$	560	0.70	0.18
3	1.108	Imidazole-2-aminovinyl-5-carboxylic acid	$C_6H_7N_3O_2$	153	2.38	0.76
4	44.600	3-Methyl-2-Methoxy pyrazine	$C_6H_8N_2O$	124	0.60	0.14
5	39.275	Arginine	$C_6H_{14}N_4O_2$	174	0.65	0.13
6	43.806	Methyl Jasmonate	$C_{13}H_{20}O_3$	224	0.71	0.08
7	45.041	Haloxazolam	$C_{17}H_{14}BrFN_2O_2$	376	0.81	0.14
8	40.199	Asparagin	$C_4H_8N_2O_3$	132	0.50	0.11
9	40.559	Carbamic acid methyl ester	$C_2H_5NO_2$	75	0.99	0.13

The GCMS study with crude methanol extract of *Elephantopus scaber* has given preliminary idea about the presence of volatile compounds present in the extract. The study result showed that the presence of the compounds such as 2-Amino-4-hydroxypteridine-6-Carboxylic acid, 25-Hydroxy-24-methylcholesterol, Imidazole-2-aminovinyl-5-carboxylic acid, 3-Methyl-2-Methoxy pyrazine, Arginine, Methyl Jasmonate, Haloxazolam, Asparagin and Carbamic acid methyl ester.

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